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INVESTIGATION OF PORCINE CIRCOVIRUS-2 TRANSMISSION THROUGH BOAR SEMEN AND ITS EFFECTS ON SEMEN QUALITY

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PROJECT TITLE

Investigation of porcine circovirus type 2 transmission through boar semen and its effects on semen quality.

This project was started January 1st, 2002.

Introduction:

Porcine circovirus-type 2 has recently been identified as the etiological agent associated with post-weaning multisystemic wasting syndrome (PMWS) in pigs worldwide. It is a pathogen which is slowly being recognized as a significant contributor to farm losses. In addition to PMWS, several other syndromes are associated with PCV2 infection which include myocarditis as well as dermatopathy and nephropathy. Rapid, accurate recognition and removal of diseased animals along with good husbandry is the only current method of controlling losses. Recently, abortions due to intrauterine infection in sows were reported in Western Canada demonstrating that the virus negatively impacts porcine reproduction and suggested a mode of transmission that could be targeted in a disease control program. Furthermore, PCV2 has recently been shown to be shed in semen. There is insufficient knowledge of the effect of PCV2 on semen quality and fertility to assess reproduction-related losses attributable to PCV2 infection, nor has the extent of horizontal transmission fully elucidated.

Objectives:

There were three goals for this project: (1) to characterize viral shedding of PCV2 in boar semen, (2) to assess the effects of PCV2 infection on semen quality, an indirect measure of fertility and (3) determine the kinetics of virus transmission in endemically infected herds.

Results:

Objective 1

All boars in this study tested were positive for PCV2 antibodies in serum by cELISA prior to initiating semen collections for this study. None of the animals within the facility presented with clinical disease associated with PMWS or PCV2-associated syndromes prior to or during the study.

PCV2 DNA was detected in semen collected from naturally infected boars from a commercial AI barn. PCV2-shedding in semen was sporadic in frequency over the period of 6 to 9.5 months, and the majority of boars with PCV2-positive semen had few (1 or 2) individual positive samples detected in completed collections. Sequential PCV2-positive semen samples were observed in boars with greater than 3 positive samples, but this observation was limited by the small number of boars in the study with multiple PCV2-positive samples.

The maximum duration of shedding from a single boar in this study was 5 non-sequential collections spanning approximately 6.5 months. The largest number of individual PCV2-positive semen samples from a single boar was 8 samples over a period of 3 months. Therefore, notable variability was observed in the duration and overall number of individual semen samples with PCV2 DNA detected. This shedding variability of PCV2 in boar semen could increase the transmission of virus from boars to PCV2-susceptible sows and fetuses if viable virus is present and individual collections were not tested for PCV2 prior to insemination.

Boars ranged from 8 to 36 months of age for the period of this study, and we observed that boars older than 16.5 months of age did not shed PCV2 DNA in semen. This observation could be critical in barns where the age of the population of boars for commercial production is less than 16.5 months, increasing the risk for PCV2 in transmission in semen.

PCV2 DNA was detected in the semen from the collections of Duroc and Landrace breeds only. Remaining breeds of Hamline, Large White, Sireline Large White and Genex Meishan were negative for PCV2 DNA, but the number of boars available for each breed tested and the total number of individual samples were not equal among breeds and collections.

Similar to PCV2, porcine reproductive and respiratory syndrome virus (PRRSV) has caused persistent infection in boars, and PRRSV DNA was detected in semen by PCR. Transmission studies have determined that exposure of gilts to PRRSV-infected semen resulted in seroconversion and isolation of PRRSV from tissues in infected gilts. Landrace, Yorkshire and Hampshire boars were infected with PRRSV experimentally and variability was observed between boar breeds in the duration of virus shed. The study suggested that Landrace boars were the most susceptible to PRRSV-shedding in semen and implicated variable host factors in PRRSV persistence and resistance.

All boars were positive for PCV2 antibodies in serum and negative for PCV2-associated diseases; therefore, shedding of PCV2 may occur in the absence of clinical disease. It is not known if PCV2 detected by PCR in semen is infectious or what quantity of the virus is present; therefore, molecular assays are an efficient detective method to determine the presence of the virus. PCV2 virus isolation from semen has not been reported. The nPCR assay used in this study was highly sensitive and the biological significance of a positive PCV2 result is unknown.

Assays less sensitive than the nPCR assay used in this study have not detected PCV2 DNA in boar semen. PCV2 shed in semen may contribute to the infection of susceptible sows and fetuses upon breeding. However, PCV2 isolation from semen, or a bioassay using PCV2-susceptible sows may be required to confirm the potential infectivity of PCV2 in semen.

Further investigation into the effects of breed and age on the shedding characteristics of PCV2 in semen is needed. The frequency of PCV2 DNA detected in semen in this study was low and sporadic, and regularly scheduled collections would assist in further discerning the frequency of viral shedding. It must be considered that boars seropositive for PCV2 may have persistent shedding of the virus in semen, and testing for PCV2 in semen may be required for commercial AI barns supplying semen to PCV2-negative sows. The significance of PCV2 DNA in semen as it relates to transmission of the virus and its effects on reproductive success is currently unknown. The results presented in this study confirm the sporadic and long-term shedding of PCV2 DNA in semen from naturally infected boars and suggest that boar age and breed may contribute to the persistence of PCV2-shedding in semen.

Objective 2

Statistical analysis of sperm morphology suggested that there was no correlation between sperm defects and virus shedding. While there may have been a trend for pigs of certain breeds to have a propensity to shed PCV-2 in semen but the limited number of animals in some breed groups and the limited number of breeds utilized in these commercial barns prevented adequate rigour to draw any conclusions.

Objective 3

Pigs tested in this study belonged to a commercial farrow-to-finish herd that have been endemically infected with PCV2 and accompanied by sporadic and low numbers of confirmed cases of PMWS or PCV2-associated diseases since 1999. There was no recognized incidence of PMWS or PCV2-associated disease during the collection period from Oct. 2002 to Mar. 2003 in pigs in this study. The 40 pigs randomly selected in this study were not segregated, but maintained in the barn with the remaining population and relocated to pens within the barn based on previously established management practices.

It was observed that at 5 (±1.5) days after birth, piglets had a high percent inhibition (PI) of PCV2-specific Ab in serum and this remained elevated until weaning at 19 days of age suggesting a transfer of maternal PCV2-specific Ab to nursing piglets. A decline in maternal PCV2-specific Ab was observed in post-weaning serum collections at 40 and 72 days of age, and the lowest average PI occurred at 72 days of age (53 days post-weaning) coincident with the first detectable PCV2 DNA by PCR. The average PCV2-specific Ab PI did not decline below the cut-off for a positive result for all collections. However, 10% and 15% of the pigs tested at 40 and 72 days of age respectively, had a PCV2-specific serum Ab decline result suggesting a wide variability in the serological positives of individual animals. Variation in the retention of maternal Ab and the immune response against PCV2 in young swine may therefore contribute to the persistence of PCV2 in pig herds, and the subsequent manifestation of PCV2-associated diseases.

Viremia (PCV2 DNA in serum) was detected followed by seroconversion (a rise in PCV2-specific Ab) in serum from pigs tested by each of 2 PCR protocols (nPCR and PCR) and cELISA respectively, from post-partum until slaughter. PCV2 DNA was detected in 71.7% of pigs tested by nPCR and in 35.9% of pigs by PCR assay at bleed 5 (172 days of age) coincident with the lowest amount of PCV2-specific antibody present in serum. Until viremia was detected at 72 days of age, PCV2 DNA was not detected in any animal up to and including bleed 4 (40 days of age) suggesting either sufficient immunological protection by maternal PCV2-specific Ab against PCV2 infection was present or the absence of exposure to PCV2 virus. Since PCV2 was present and has circulated within animals in the barn used in this study as determined by serological, molecular and clinical diagnosis, it is clear that pigs in this study were exposed to PCV2 prior to 72 days of age. PCV2 can be detected in ocular, nasal and fecal secretions supporting horizontal transmission of the virus from sows to nursing piglets, and between littermates. It is postulated that vertical transmission of PCV2 can occur in fetal pigs as PCV2 has been isolated from foetal pig tissues from abortions and stillbirths, and PCV2 DNA has been detected in semen used to artificially inseminate sows in this barn (unpublished data). Infectious PCV2 transmission via semen or colostrum has not been reported, but it is a potential means of transmission to foetal or nursing piglets. Data from this study suggests that exposure to infectious PCV2 may have occurred prior to 40 days of age, but viremia was not established in piglets possibly due to the conferred protection by high levels of circulating PCV2-specific Ab.

Seroconversion (an increase in PCV2-specific Ab PI following a decline) was observed following bleed 5 (72 days of age) and the amount of PCV2-specific Ab remained high in subsequent collections (bleed 6 to 8) thereafter to the end of the study. The sequence of these events suggests that PCV2 viremia is established concurrent with waning PCV2-specific serum Ab, however, PCV2 DNA was periodically detected in serum until the time of slaughter in the presence of high amounts of PCV2-specific Ab which suggests that serum Ab may not be protective once an infection is established. An alternate explanation is that physiological changes associated with weaning triggers intermittent virus production and is independent of serum Ab concentrations.

PCV2-specific serum Ab was not quantified in this study, rather, a cELISA was used and Ab measured by percent binding and this method does not provide a measure of concentration or titre. PI is qualitative and therefore can be discussed only in terms of relative antibody amounts for comparison between pigs and over time.

PCV2 DNA was intermittently detected in serum beginning at bleed 5 (72 days of age) until slaughter (bleed 8; 156 days of age) by the nPCR or the PCR assay in the presence of PCV2-specific serum Ab. Therefore, animals with persistent infections provide a constant source of PCV2 for naïve pigs, as well as pigs that clear the infection.

PCR is a sensitive molecular diagnostic tool that can detect PCV2 at low concentrations in serum but does not provide information on quantity or infective capability of the virus. PCR assays used in this study had different detection limits. A greater number of samples were positive for PCV2 DNA in serum when tested with the nPCR protocol compared to the PCR protocol. Although both statistically significant by Wilcoxin Rank Sum, a stronger association between the decline of PCV2-specific antibody PI in the blood collections preceding the detection of viremia (bleed 3 and 4) and a positive PCR result (bleed 5) was observed in the less sensitive PCR assay compared

with the nPCR assay. While the highly sensitive nPCR assay may be useful as a diagnostic tool to detect very low amounts of PCV2 virus, the results from a non-nested PCR may be more useful to determine an amount of virus that indicates active viral replication and therefore have greater significance for disease, or the potential for viral shedding and the spread of PCV2 between animals.

PCV2-associated diseases have significant impacts on swine populations worldwide. Research examining the epidemiology of PCV2 is limited in herds endemic with the virus and with accompanied sporadic or low incidences of overt PCV2-related disease. Epidemiological studies of PCV2 infection will contribute to the development of vaccination strategies and understanding the pathogenesis of PCV2 and PCV2-associated diseases.